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Intensity and temporality of airborne *Quercus* pollen in the southwest Mediterranean area: Correlation with meteorological and phenoclimatic variables, trends and possible adaptation to climate change

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ABSTRACT

This paper deals with aerobiological analyses realised during last 25 years in the atmosphere of Malaga, a coastal city located in the southwest of the Mediterranean Basin. Air sampling was performed by means of 7-day recording volumetric pollen and spore traps, and pollen grains were counted with the aid of a light microscope, according to the methodology proposed by the Spanish Aerobiology Network. Pollen data were expressed as number of pollen grains per cubic metre of air. A peak in *Quercus* pollen production approximately every four years was detected, coinciding with drought periods. Although the natural vegetation of the studied area has been altered by urban growth and reforestation with pines, it is still represented by a disperse natural population of cork oak, holm oak and kermes oak (predominantly located to the northwest and northeast of the city). In this period the seasonal behaviour of anemophilous pollination of *Quercus* was studied, along with the relation between its intensity (pollen production) or temporality (phenophase of flowering) and meteorological or climatic variables. Also a study of trends in production and phenology of flowering was carried out. The annual intensity of anemophilous pollination of *Quercus* was significantly associated with the meteorological conditions of each spring, with the same parameters involved and in the same way as were seen on the daily and weekly scale (positive correlation with temperature and insolation, and negative with precipitation and relative humidity). The tendency for temperature and atmospheric aridity to increase is probably the cause of the trend observed in the spring *Quercus* pollen production to increase in the western Mediterranean. The temporality of *Quercus* anemophilous pollination (start date, peak date, end date and duration) changes each year and is positively associated with accumulated temperature and sun hours from 1st January until the dates in question. An accumulation of approximately 796 °C above the 9 °C threshold temperature from 1st January is necessary to trigger the start of the flowering period. We conclude that the effect of climatic change is mainly reflected in the pollination intensity of woody anemophilous species, which, in turn, have adapted their flowering time (phenology) to climate change. It is important to remember that climate change is leading to more arid conditions and that Mediterranean plants are adapted to this macrobioclimate (Mediterranean), which is characterized by a long dry period and high temperatures.

1. Introduction

Mediterranean forest and "dehesas" (traditional, semi-natural, man-made systems present in the Iberian Peninsula) (Gómez-Casero et al., 2007) represent the natural and seminatural characteristic vegetation of Andalusia (south of Spain). The dominant tree species of these forests and dehesas mainly belongs to *Quercus* genus. These natural forests are characterized by high biological diversity (flora and fauna) and constitute one of the most diverse ecosystems in Europe, hence the interest in conserving these forests. In addition, the presence of trees of *Quercus* species in these forests indicate ecological maturity, as they are responsible for maintaining the physical-chemical and microclimatic characteristics of these ecosystems.

The Mediterranean Basin is considered as a hot spot of biodiversity worldwide (Médali and Quézel, 1999). The south of the Iberian Peninsula is characterized by its strategic settingt, between two seas and between two continents, with a particular geological and biological history that has decisively influenced the present day diversity observed. The flora and vegetation of this part of the western Mediterranean is a consequence of the adaptation to climatic, geological,

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orographic, topographic and environmental changes that it has been exposed to for thousands of years. For this reason, the south of the Iberian Peninsula contains examples of plant species of very different origins. These include Mediterranean perennial species such as the oak (*Quercus rotundifolia* Lam.), which lives in thermomediterranean and mesomediterranean thermotypes, but also in the supramediterranean where it forms mixed forests with maples and gall oak (*Quercus faginea* Lam.) (Cabezudo and Pérez Latorre, 2004). Another Mediterranean perennial species is the cork oak (*Quercus suber* L.) which lives in the same termotypes as oak, but with subhumid ombrotypes and acid soils. *Quercus pyrenaica* Willd. is a deciduous species that lives in the mountain areas with cold winters and acid soils. *Quercus canariensis* Willd. is a relict deciduous species of laurel subtropical forests (currently protected) which originated in the Tertiary in areas exposed to mountain fog. One endemic species is threatened, *Quercus alpestris* Boiss., cataloged by UICN Red List as endangered (EN) (Cabezudo et al., 1999). It lives in the high-mountain acciculitic forests (fir forest) that survived glaciations. As consequence of recent climate change, distribution of these *Quercus* species are expected to shift.

Through the study of the presence, quantity and production dates of *Quercus* pollen in the atmosphere, changes in distribution, phenology and ecological conservation state of Mediterranean forest can be detected. The importance of detecting such changes lies in the high economical interest of these vegetal formations for the timber industry, agriculture and animal husbandry. The most valued pig herds are fed with acorns produced by *Quercus* species, as are sheep and cattle, which produce high quality meat. Acorns production for a particular year can be estimated from the quantity of pollen produced and, therefore, agricultural production (both crops and animals) can also be estimated (García-Mozo et al., 2002, 2008, 2010; Gómez-Casero et al., 2004, 2007; Jato et al., 2007; Hernández-Ceballos et al., 2011, 2015).

Quercus pollen is considered as a moderate respiratory allergies cause in many areas of Europe because the high amounts of pollen which this anemophilous taxon produce. Furthermore, the flowering periods of the different species of the genus are overlapped in time, which produce allergenic symptoms in population during long periods of time. A cross reactivity process with other pollinic types such as *Alnus, Betula, Castanea, Olea* and Poaceae, has also been detected. This process increases the allergenic potentiality of this pollen type (Recio et al., 1999; García-Mozo et al., 2002; Rodríguez-Rajo et al., 2005; Jato et al., 2007).

The great abundance of these taxa increases their impact on allergy sufferers, especially people who live or work in areas covered with natural or semi natural vegetation such as holm-oak forests, cork oak forests and dehesas (García-Mozo et al., 2006a). The study of the seasonal behaviour and the different ways to forecast variations of *Quercus* pollen levels will provide useful information for treating allergy symptoms a few days before exposure to the allergen or even enable people to avoid them. Many research teams are engaged in detecting the pollen types which are present in the atmosphere at any moment of the year, designing models and analyzing the causes of the variations in airborne pollen levels (Jato et al., 2002; Rogers et al., 2006; Docampo et al., 2007; Gómez-Casero et al., 2007; Martínez-Bracero et al., 2015; Rojo et al., 2015).

The information obtained through a study of the starting date and peak date of the atmospheric main pollen season of some taxa during long series of years can also be used as bioindicator of climate change. Some authors have used *Quercus* pollen levels as a bioindicator for climate change (Gómez-Casero et al., 2007; Tormo-Molina et al., 2010). Any influence of global warming on *Quercus* species phenology is of great relevance because of the great economic and ecological importance of this taxon in the Mediterranean area (García-Mozo et al., 2008).

An increase of 1.5 °C per year in the minimum temperature has been detected in Spain, an increase that is more intense in the south of Spain, where precipitation has decreased a lot (Gordo and Sanz, 2005; García-Mozo et al., 2010). Climatic factors have a strong influence over airborne pollen concentration, underlining the effect of climate change on atmospheric pollen concentration (García-Mozo et al., 2002, 2008; Peñuelas et al., 2004, 2009; Rogers et al., 2006; Hedhly et al., 2009; Recio et al., 2009; Tormo-Molina et al., 2010; Guo et al., 2014).

Over the last 50 years, the majority of the Mediterranean macrobioclimate plants have shown alterations in their phenological behaviour (Peñuelas et al., 2002; Wolkovich et al., 2012). This change has been detected with much more intensity in the winter and early spring phenophases since it is the temperatures of these seasons that have changed the most during the recent years (Bertin, 2008). In general, the flowering phenophase of *Quercus* species has been brought forward (Peñuelas et al., 2002). As regards atmospheric pollen studies, some authors have detected a trend towards an earlier start and earlier end of the main pollen season in Mediterranean macrobioclimate areas. This behaviour slightly differs from that temperate macrobioclimate species which show a tendency to a later start and earlier end of the pollen season. This trend is especially pronounced in anemophilous trees, which flower in early spring. An example of such trees members of the *Quercus* genus, whose reproductive buds development is very sensitive to temperature. Moreover, an earlier start to the spring season as a result of increased temperatures also affects vegetative growth and other late-flowering species (Rogers et al., 2006; Tormo-Molina et al., 2010). An increase in temperature and reduced rainfall have been identified as the causes of the increase in airborne pollen quantity during the months with the highest concentrations (Wolkovich et al., 2012). However, despite these general trends, some studies have not found any significant change (Bertin, 2008).

In Malaga (a Spanish city located on the western Mediterranean coast) the temperature has increased by 0.06° C per year since the 1970s (see Fig. 1 in Recio et al., 2010). In the western Mediterranean region changes in rainfall have been observed (Maheras 1988; Piervitali et al., 1997; Esteban-Parra et al., 1998; De Luís et al., 2000). As regards the wind, although this is one of the variables that most influences pollen registers, few studies have looked at any trends in this climatic variable. In Malaga, there has been a very significant decrease in calm periods since the 1970s and an increase in the frequency of Levant winds (from the second quadrant, off the sea) (Recio et al., 2010). The uninterrupted aerobiological sampling carried out in Malaga during the last 25 years permits us to make some interesting observations about global change. Therefore, the objectives of this study were: to determinate the seasonal pollen behaviour, detect correlations between pollen concentrations and meteorological variables, and identify trends in *Quercus* pollination intensity and time. We use "pollination" to refer the final potential effect in aerobiological trajectory (deposition) (Spieksma, 1992).

2. Materials and methods

2.1. Study area

The city of Malaga is situated in the south of the Iberian Peninsula (36°47′N, 4°19′W), on the western Mediterranean coast, and lies in an alluvial plain that is partially surrounded by mountains. The climate is Mediterranean (Martonne, 1964), the mean annual temperature being 18 °C, with a mean maximum of 22.8 °C and mean minimum of 13.8 °C. Annual rainfall is 575 mm on average, falling mainly in autumn (October to December) and winter (January to March), while the summer (June to September) is the driest period. Due to the city's orography and geographical situation, the dominant winds have a SE (blowing off the sea) and NW (from the interior) component. Calms represent 14% on average, while the predominant winds have a NW component (blowing off the land) and a SE component (blowing off the sea), known locally as "terral" and "levante" winds, respectively (Domínguez Rodríguez 1984; Viedma Muñoz 2001, 2002).

The natural vegetation of the area has been much altered by the

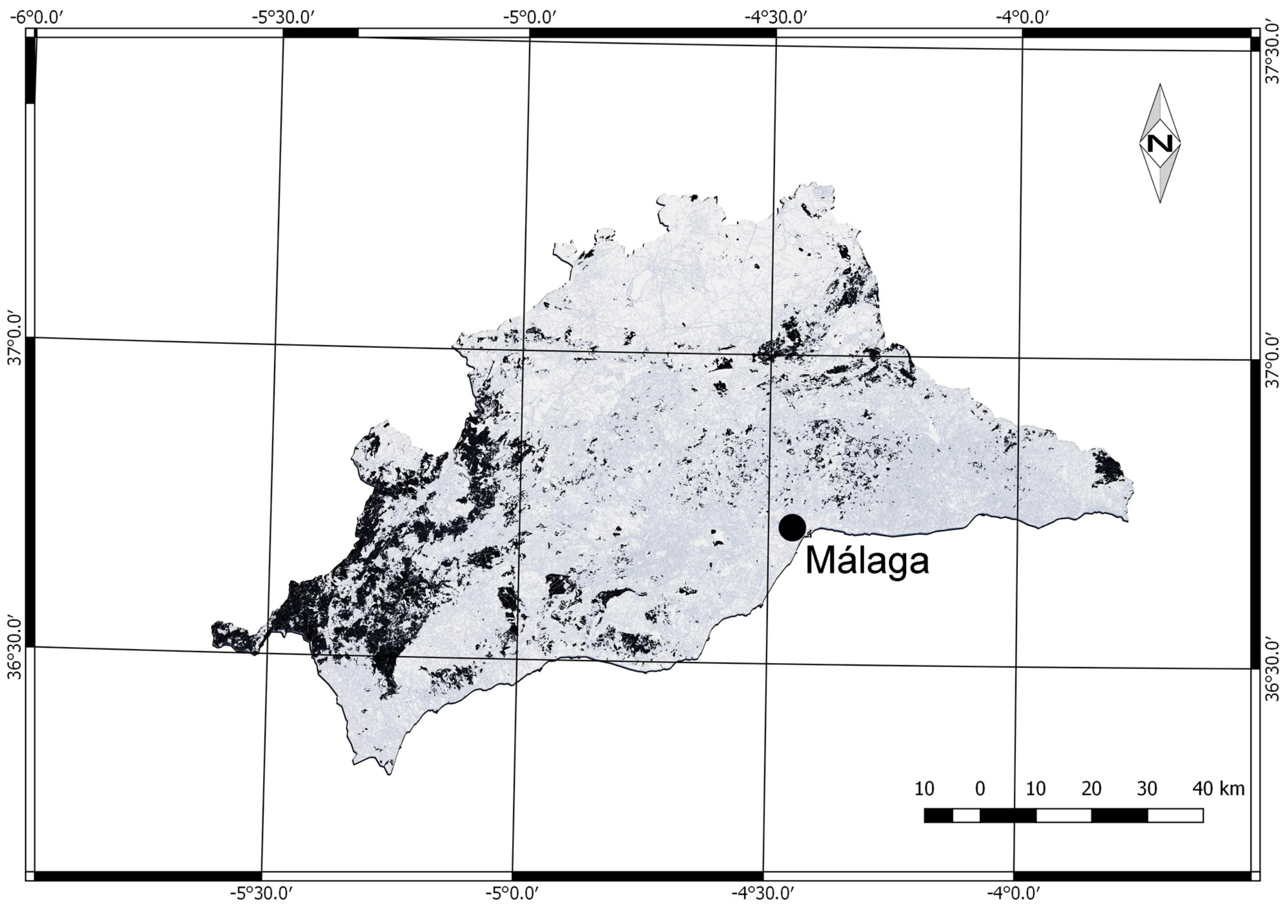

Fig. 1. Real distribution of *Quercus* forest (black areas) in the province of Malaga, processed from Andalusia 2011 SIOSE.

urban growth, which has led to an increase of herbaceous taxa at the expense of shrub and arboreal vegetation accompanied by a substantial increase in ornamental flora. The predominant crops near the city are citrus, olive, almond, vineyards, traditional Andalusian crops and sugar cane. The natural vegetation is represented by disperse populations of cork oak, holm oak and kermes oak, but in many cases it has been replaced by reforestation pines (Recio et al., 1998). This natural vegetation is predominantly located to the northwest and northeast of the city, as can be observed in the real vegetation map of the province (Fig. 1).

2.2. Atmospheric pollen data

Quercus pollen grains are stenopalynous, which means that they have an almost identical morphology in all *Quercus* species (10–50 µm in diameter). However, the size of the pollen grains can change slightly between the different species. In this respect, *Quercus coccifera* L pollen grain is the smallest of the genus and *Quercus suber* L the largest (Recio et al., 1999; Jato et al., 2002; García-Mozo et al., 2002, 2006a, 2010; Gómez-Casero et al., 2004). For the purpose of the present study, all the species are identified as the same pollen type due to the difficulty involved in differentiating the grains of each species with optical microscopy.

Air sampling was performed by means of 7-day recording volumetric pollen and spore traps (Hirst, 1952) operating uninterruptedly from 1991 and placed on the roof of a building in the Teatinos Campus of the University of Malaga to the west of the city. The sampler was situated about 15 m above the ground level, in an open space without nearby buildings that could obstruct the free circulation of air, and calibrated to manage a flow of 10 L of air per minute, thus matching the human breathing rate. In the trap, pollen grains (and others solid particles, biotic or not) impact on a cylindrical drum covered by a Melinex™ film coated with an adhesive substance. The drum was changed weekly and the exposed tape cut into seven pieces of 48 mm, which were mounted on separate glass slides, each one corresponding to 24 h of sampling, since the drum runs at 2 mm per hour by means of a clockwork mechanism. Glycerine jelly stained with fuchsine was used as mounting medium (Galán et al., 2007).

Pollen grains were counted with the aid of a light microscope, according to the methodology proposed by the Spanish Aerobiology Network (REA, in its Spanish abbreviation) (Domínguez 1992). This consisted of reading four longitudinal sweeps per slide at magnification of x400 (Domínguez et al., 1991). Finally, daily values were expressed as number of pollen grains per cubic metre of air (daily mean values) (Galán et al., 2007).

To establish the Main Pollen Season (MPS) the method proposed by Nilsson and Persson (1981) was used. This selects the days that, taken together, represent 90% of the annual total, beginning the day that the accumulated value reaches 5% of the annual total and ending on the day that 95% of the annual total is reached. The day in which the maximum pollen concentration is registered is denominated peak, which breaks the MPS into two periods: pre-peak and post-peak (Toro et al., 1998).

The dates of the beginning and end of the MPS (days counting from 1st January) and its length in days were used to characterise the phenological behaviour of atmospheric pollen. The annual pollen integral (annual pollen index) and the spring pollen integral (spring pollen index) were used as indicators of pollen production. The annual pollen index is the annual sum of daily average pollen concentrations (Galán et al., 2017). The spring pollen index is the sum of daily average pollen concentrations from 1st April to 30th June.

2.3. Meteorological and phenoclimatic data

The meteorological data used were the mean, maximum and minimum temperatures (degrees Celsius), sunshine hours, rainfall (millimetres), relative humidity (percent), wind speed (kilometres per hour), frequency of wind from the quadrants centred in NE (first), SE (second), SW (third) and NW (fourth), and the frequency of calm weather. Daily, weekly and annual values of these variables were used. The meteorological data were supplied by the Territorial Meteorological Centre of Eastern Andalusia and were recorded at Malaga Airport, which is located five kilometres south of the sampling site.

Annual values of accumulated daily sun hours, daily mean temperature, daily maximum temperature, daily minimum temperature and daily precipitation since 1st January to the start, peak and end of the MPS were used as phenoclimatic variables.

Some *Quercus* taxa require a dormancy period in winter followed by a warming or heat-accumulating period before spring flowering (Jato et al., 2002, 2007; Rodríguez-Rajo et al., 2005). This bud dormancy period in unfavorable conditions is also called quiescence. During this period the plant produces hormonal changes in its buds that will allow the subsequent growth. Once all the cooling requisites have been achieved, a period of “imposed dormancy” is required. In this period the plant accumulates heat to start its reproductive activity (Aron and Gat, 1991; Galán et al., 2001; Guo et al., 2014).

When a given temperature is accumulated, the floral buds growth is induced. The required accumulated temperature varies depending on the species and the climatic conditions. If these accumulated heat requirements after dormancy are known, it is possible to predict the start of the flowering phenophase and the start of the MPS (Galán et al., 2001; García-Mozo et al.,2002; Bertin, 2008). According to some authors, the shorter the dormancy period the longer the heat-accumulating period required (Aron and Gat, 1991; Rodríguez-Rajo et al., 2005). If high temperatures are registered during the dormancy period, dormancy may be delayed, which also delays flowering and the start of the MPS (Galán et al., 2001; Jato et al., 2002; Cook et al., 2012; Ziello et al., 2012; Guo et al., 2014).

To calculate the accumulated heat from the end of the dormancy period until the start of the MPS two different methods were used:

● Degree-days (°D): Sum of the difference between daily mean temperature (T_{mean}) and a threshold temperature (T_{thr}).

$$°D = \Sigma(T_{mean} - T_{thr})$$

● Heat Requirement (HR): Sum of the difference between daily maximum temperature (T_{max}) and a threshold temperature (T_{thr}) which allows phenological activity.

$$HR = \Sigma(T_{max} - T_{thr})$$

The threshold temperature changes in each locality depending on the annual mean temperature. The most appropriate threshold temperature for Malaga is 9 °C according to a previous study (García-Mozo et al., 2002; Jato et al., 2002, 2007).

There are also different criteria to determinate the heat-accumulating period:

- Accumulated temperature from 1st January of each year until the start of the MPS (García-Mozo et al., 2002).
- Accumulated temperature from the date on which the lowest daily mean temperature of winter is registered and from which a trend change in the temperature is detected, until the start of the MPS (Jato et al., 2002). A graphic representation of the daily mean temperatures of each year from October of the previous year to the end of the year was made to calculate this date. The chosen date was the day with the lowest daily mean temperature from the beginning of this period until the start of the MPS.
- Accumulated temperature between the date when a daily mean temperature is higher than the 9 °C threshold temperature and the start of the MPS (Galán et al., 2001; García-Mozo et al.,2002).

For each combination of method and criterion, the mean, standard deviation and variation coefficient were calculated.

2.4. Statistical analysis

Correlations with meteorological variables were made to understand their influence on the pollen concentrations detected using the IBM SPSS Statistics 21 package. As the data did not follow a normal distribution according to a Kolmogórov-Smirnov test, a non parametric statistic was chosen. Spearman correlations were run with daily and weekly values using individual years and all the 24 studied years together. Furthermore, each part of the MPS (full MPS, pre-peak period and post-peak period) was considered.

Only the years with a pre-peak period of 4 or more weeks with pollen levels higher than or equal to 10 pollen grains/m^3 of air were taken into account for the weekly data. These years were the only ones with a sufficiently high number of replies to avoid error or statistical noise.

Correlations were also made using annual phenological values such as the start, peak, end date and length of the MPS, the peak concentration, the annual pollen index and some phenoclimatic variables: temperature, sun hours and precipitations accumulated since 1st January, vapor pressure deficit (VPD) in autumn of the previous year, VPD in winter, temperatures, sun hours, wind speed and precipitation in spring months, degree days and heat requirements.

The annual pollen and climatic data were fitted to simple linear regression lines to observe trends. The slopes of the regression equations, the determination coefficients (R^2) and significance levels (p) were studied. This study only considers as significant the regression lines whose fitted points as determined from the R^2 value, showed a p value of ≤ 0.05. To evaluate the relationship between the annual values of climatic and pollination variables, Spearman correlations were calculated.

3. Results and discussion

3.1. Seasonal behaviour of Quercus airborne pollen

Quercus pollen represents approximately 14% of the annual total pollen registered in Malaga atmosphere, although the exact percentage varies substantially from one year to another. The highest percentage of *Quercus* pollen in this respect was registered in 2005 (23%), while the proportion in 1996 was the lowest (4%). The year in which the highest amount of *Quercus* pollen was registered was 2005 with 9643 grains, and the year with the smallest amount was 1992, with 1925 grains. *Quercus* pollen is registered in the Malaga atmosphere almost throughout the year, although most (almost 98%) is registered in March, April, May and June (Table 1).

The maximum daily values exceeds 200 grains/m^3 in many cases (Fig. 2) which matches the high level rank according to the REA (Galán et al., 2007). The daily maximum of each year changes in quantity and date. The peaks of daily concentration varied between 125 grains/m^3 (year 1992) and 1578 grains/m^3 (year 1997) during the period studied (Table 2). The year with the earliest peak was 1997 (25th March), while the year with the most delayed one was 2003 (20th May). It is frequent to register more than one concentration peak in a same year. This behaviour and its relation with meteorological variables has been discussed in the next section.

Graphs with weekly values have been elaborated (Fig. 3) with the aim of reducing the statistical noise of daily data and to better understand the seasonal behaviour of *Quercus* pollen in the atmosphere of Malaga. The earliest maximum peaks were registered in the week 12 (end of March) and the latest in the week 21(end of May). The

Table 1
Annual and monthly total and mean of airborne pollen *Quercus* (pollen grains * day/m^3) registered in the atmosphere of Malaga during the studied period (1992–2015). The Annual (or monthly) Pollen Index (API) is obtained by summing the average daily concentration over the year (or month).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	API
1992	0	0	200	904	736	65	11	2	1	2	1	3	1925
1993	2	0	47	2146	461	377	20	4	8	5	1	2	3073
1994	4	4	601	2092	449	35	9	0	1	0	6	3	3205
1995	11	6	282	1856	926	141	23	2	0	0	0	0	3246
1996	3	59	51	807	554	162	5	0	2	0	3	1	1647
1997	0	4	5425	1669	837	58	5	2	0	5	9	2	8014
1998	5	5	502	3350	601	398	20	5	2	0	2	0	4891
1999	1	1	114	4430	1150	104	7	7	10	22	72	23	5940
2000	4	10	1341	726	457	131	5	1	2	0	0	2	2679
2001	2	6	1418	5903	658	103	21	3	0	9	29	4	8156
2002	1	5	200	2209	2549	169	13	3	2	5	60	4	5220
2003	13	10	79	971	2118	102	32	13	0	0	1	16	3355
2004	15	1	348	1333	751	459	20	10	3	3	2	7	2952
2005	7	3	60	6333	3036	150	22	10	17	1	2	2	9643
2006	1	3	138	1885	721	28	2	1	0	0	0	3	2782
2007	74	23	850	1268	3044	692	38	18	0	4	1	0	6012
2008	4	7	1732	1560	1415	569	45	10	10	19	23	9	5403
2009	2	0	71	6172	1307	242	61	19	24	11	16	4	7929
2010	2	2	196	701	2907	587	40	12	11	8	9	5	4480
2011	3	25	684	2175	678	168	51	20	1	3	0	3	3811
2012	5	18	62	2332	2646	535	115	31	24	17	80	19	5884
2013	24	14	107	1208	1882	293	43	27	14	17	16	15	3660
2014	25	5	1002	3396	2838	243	55	46	3	0	12	31	7656
2015	58	24	264	1549	1706	137	25	12	9	10	8	18	3822
Mean	11	10	657	2374	1434	248	29	11	6	6	15	7	4808

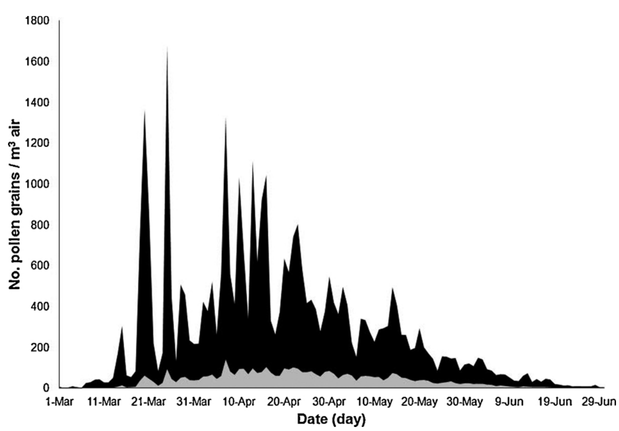

Fig. 2. Evolution in the daily mean concentrations of airborne *Quercus* pollen during the years studied (1992–2015). Average (grey area) and maximum (black area) daily values.

reduction in statistical noise allows the different pollen concentration peaks registered in a same year to be distinguished, as occurs in the years 1992, 1996, 2002, 2003, 2004, 2007, 2012 and 2014. *Quercus* populations or species bloom earlier or later depending on their geographical distribution. The populations closest to the coast flower earlier while those of mountainous areas and higher latitudes bloom later (whether of the same or different species). The pollen of different species of *Quercus* is morphologically similar and can not be distinguished. Thus, airborne pollen from mountainous areas can reach the coast by long distance transport, when flowering at the coast has finished. Sometimes long distance pollen transport may be responsible for the appearance of pollen concentration peaks which do not match the flowering period in the sampling site. This effect is responsible for the several concentration peaks that appear in a same MPS (Hernández-Ceballos et al., 2011; Rojo et al., 2015). Sometimes populations of *Quercus rotundifolia* and *Quercus suber* bloom twice a year: during spring (the greatest bloom) and also in autumn (a second, less intense bloom), as detected in early November 1999. This phenomenon was often observed *in situ*, in the field.

The Main Pollen Season length was not homogeneous during the period studied (Table 2). The shortest duration registered was in 2005 (39 days) and the longest duration in 2008 (90 days). The Main Pollen Season also showed variations in its start and end dates due to the influence of climatic variables. Those influences would be discussed later.

Table 2
Start, peak, end (dates) and duration (days) of the MPS, and peak day concentration (pollen grains/m^3) for *Quercus* in Malaga during the studied years.

Year	Start Date	Peak Date	End Date	MPS Duration	Peak Value
1992	24-mar	10-may	30-may	67	125
1993	1-apr	9-apr	15-jun	75	346
1994	22-mar	7-apr	20-may	59	261
1995	28-mar	20-apr	1-jun	65	409
1996	26-mar	17-apr	4-jun	70	225
1997	15-mar	25-mar	13-may	59	1578
1998	24-mar	8-apr	13-jun	81	419
1999	11-apr	13-apr	27-may	46	1016
2000	25-mar	28-mar	1-jun	68	455
2001	21-mar	7-apr	15-may	55	1189
2002	3-apr	26-apr	31-may	58	350
2003	2-apr	20-may	31-may	59	254
2004	22-mar	7-apr	13-jun	83	255
2005	12-apr	17-apr	21-may	39	939
2006	31-mar	20-apr	22-may	52	256
2007	24-mar	14-apr	10-jun	78	332
2008	17-mar	26-mar	15-jun	90	402
2009	2-apr	7-apr	31-may	59	1184
2010	1-apr	3-may	8-jun	68	431
2011	28-mar	10-apr	8-jun	72	937
2012	11-apr	14-may	29-jun	79	420
2013	5-apr	11-may	18-jun	74	232
2014	24-mar	30-apr	2-jun	70	462
2015	29-mar	19-apr	3-jun	66	313

MPS = Main Pollen Season.

3.2. Correlations between daily and weekly values of Quercus airborne pollen and meteorological variables

In general, the correlation coefficients and significance levels were greater for pre-peak periods than post-peak periods (Table 3), such variations depending on the considered period having been detected in

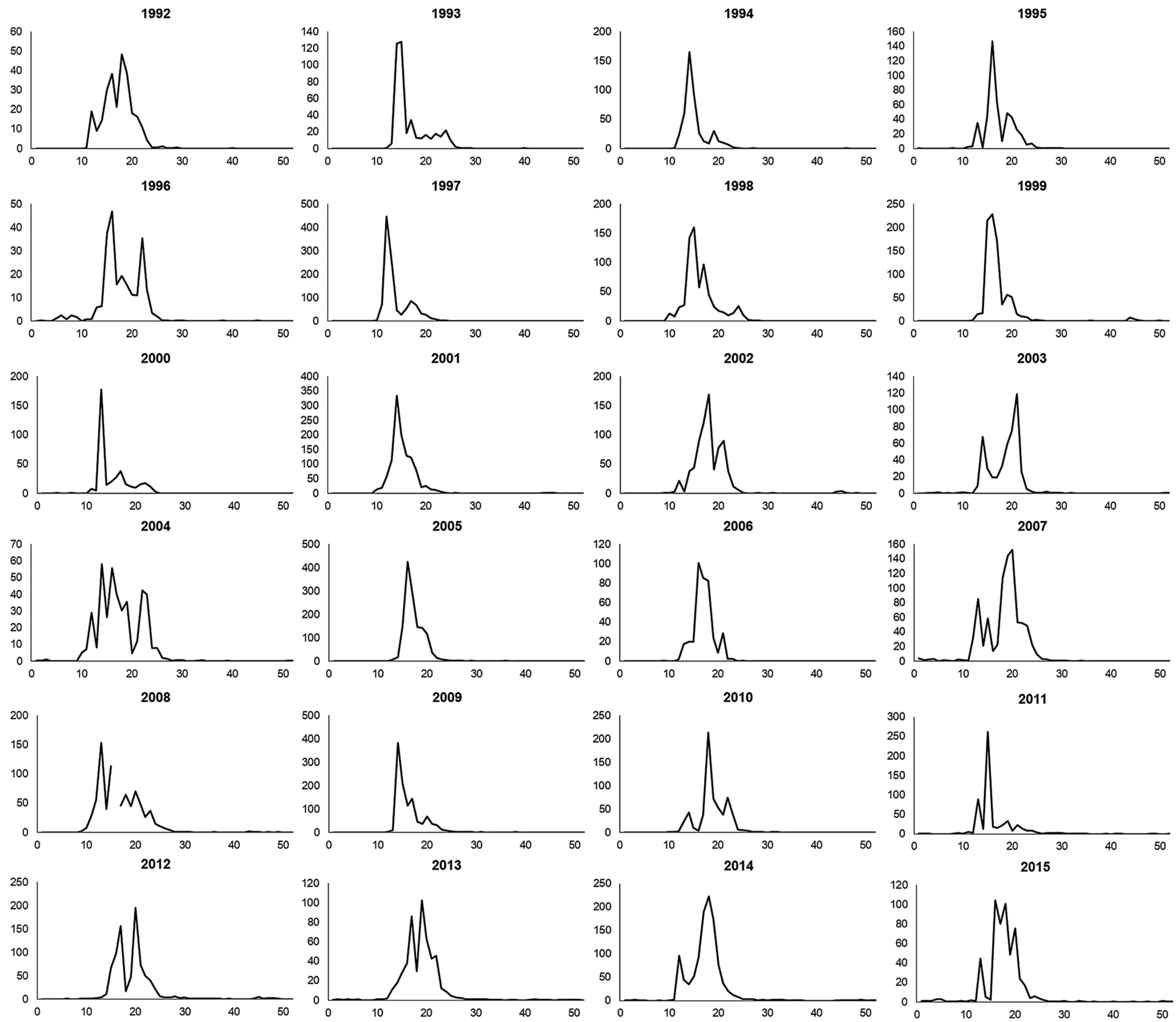

Fig. 3. Evolution in the weekly mean concentrations of airborne *Quercus* pollen during the years studied (1992–2015). Vertical axis labels: pollen grains/m^3 air. Horizontal axis labels: week.

Table 3
Spearman correlation coefficients (r) obtained between mean *Quercus* pollen concentration and several meteorological variables using daily and weekly values of all the studied period in Malaga.

	Daily values			Weekly values	
	MPS (n = 1606)	Pre-peak (n = 503)	Post-peak (n = 1123)	MPS (n = 383)	Pre-peak (n = 85)
Sunshine Hours	0.234***	0.334***	0.202***	0.175***	0.456***
Rainfall	−0.250***	−0.254***	−0.260***	−0.097	−0.291**
Relative Humudity	−0.378***	−0.457***	−0.353***	−0.258***	−0.417***
Mean Temperature	0.237***	0.460***	0.155***	0.086	0.483***
Maximum Temperature	0.316***	0.525***	0.234***	0.124*	0.517***
Minimum Temperature	0.084***	0.213***	0.027	0.014	0.367***
Mean Wind Speed	0.367***	0.353***	0.390***	0.194***	0.309**
Frequency of 1 st Q (NE) Wind	−0.058*	−0.176***	−0.006	−0.019	0.017
Frequency of 2nd Q (SE) Wind	−0.313***	−0.428***	−0.264***	−0.242***	−0.153
Frequency of 3rd Q (SW) Wind	−0.050*	−0.034	−0.072*	0.094	0.020
Frequency of 4th Q (NW) Wind	0.335***	0.459***	0.286***	0.258***	0.213*
Frequency of Calm	−0.209***	−0.249***	−0.200***	−0.145**	−0,323**

MPS = Main Pollen Season. Q = quadrant. Significant levels: p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***).

other studies (Recio et al., 1996; García-Mozo et al., 2006a; Rojo et al., 2015), which suggested that the meteorological variables related with pollen concentrations depend on the period considered.

Precipitations had a negative correlation with pollen concentration due to the rain washing effect in the atmosphere (Gómez-Casero et al., 2004; García-Mozo et al., 2006a; Docampo et al., 2007; Tormo-Molina

Table 4
Spearman correlation coefficients (r) obtained between annual values of climatic variables and *Quercus* pollination phenological variables.

	Ac. Sun Hours	Start Ac. Sun Hours	Peak Ac. Sun Hours	End Ac. Sun Hours
MPS Start Date		0.629***		
Peak Date			0.840***	
MPS End Date				0.717***
MPS Duration				0.594**
Peak Value			−0.243	
Anual Total Pollen	0.426*	0.183	−0.003	0.162

	Ac. Rainfall	Start Ac. Rainfall	Peak Ac. Rainfall	End Ac. Rainfall
MPS Start Date		0.393		
Peak Date			0.059	
MPS End Date				0.082
MPS Duration				0.093
Peak Value			−0.21	
Anual Total Pollen	−0.17	−0.26	−0.114	−0.25

	Ac. Mean Temp.	Start Ac. Mean Temp.	Peak Ac. Mean Temp.	End. Ac. Mean Temp.
MPS Start Date		0.814***		
Peak Date			0.915***	
MPS End Date				0.931***
MPS Duration				0.855***
Peak Value			−0.449*	
Anual Total Pollen	0.443*	0.243	−0.15	−0.043

	Ac. Max. Temp.	Start Ac. Max. Temp.	Peak Ac. Max. Temp.	End. Ac. Max. Temp.
MPS Start Date		0.877***		
Peak Date			0.949***	
MPS End Date				0.945***
MPS Duration				0.865***
Peak Value			−0.410*	
Anual Total Pollen	0.502*	0.245	−0.096	−0.07

	Ac. Min. Temp.	Start Ac. Min. Temp.	Peak Ac. Min. Temp.	End Ac. Min. Temp.
MPS Start Date		0.549**		
Peak Date			0.833***	
MPS End Date				0.878***
MPS Duration				0.782***
Peak Value			−0.430*	
Anual Total Pollen	0.410*	0.127	−0.156	−0.057

MPS = Main Pollen Season. Ac. = accumulated during the completed year. Start Ac. = accumulated until the beginning of the main pollen season. Peak Ac. = accumulated until the peak day. End Ac. = accumulated until the end of the main pollen season. Temp = temperature. Min = minimum. Max = maximum. Significant levels: $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***).

et al., 2010; Rojo et al., 2015). A similar effect can be attributed to relative humidity which is responsible for the binding of particles in suspension in the atmosphere and their precipitation (Rojo et al., 2015).

Temperature (mean, maximum and minimum) showed a positive correlation with pollen concentration. This correlation is mentioned by other authors who identified temperature as one of the meteorological variables with the greatest influence over pollen concentration (Recio et al., 2000; Galán et al., 2001; García-Mozo et al., 2002, 2006a, 2008, 2010; Jato et al., 2002, 2007; Peñuelas et al., 2002; Gordo and Sanz, 2005; Rodríguez-Rajo et al., 2005; Bertin, 2008; Tormo-Molina et al., 2010; Hernández-Ceballos et al., 2011, 2015; Cook et al., 2012; Wolkovich et al., 2012; Cariñanos et al., 2014; Rojo et al., 2015). The positive correlation detected between pollen concentration and meteorological variables such as temperature and sun hours may be explained by the drying and dehiscence effect on anthers (Rojo et al., 2015).

Wind speed showed a significant positive correlation with pollen concentration due to a pollen transport effect. For the same reason, calm showed a significant negative correlation. In coastal cities like Malaga, wind direction can considerably influence in the airborne pollen register. In our case, there was a positive correlation between wind direction and pollen levels when the wind blows from the land (mainly from the 4th quadrant), and a negative correlation when the wind blows from the sea (2nd and 3rd quadrants). Such correlations have been detected for other taxa such as olive (Recio et al., 1996) and by other authors (García-Mozo et al., 2006a). The very low coefficients observed with the first and third quadrants would be due to the low frequency of these winds (the main winds in Malaga blow from the NW and SE) (Recio et al., 1996, 2009).

As regards the weekly data the correlations were very similar. The higher correlation coefficients were due to the reduction of statistical noise.

3.3. Relation between intensity and temporality of *Quercus* pollination and climatic variables

As regards to the correlations with annual values (Table 4) a significant positive correlation was detected between accumulated sun hours and the total annual pollen (intensity of pollination). The same occurred for accumulated temperatures. The findings support the hypothesis concerning the influence of the temperature and sun on the flower maturation process and on the number of flowers with mature anthers in the masculine catkins.

Table 5
Spearman correlation coefficients (r) obtained between annual values of *Quercus* spring total pollen (spring pollen index) and several climatic variables before and during the spring.

		Spring Pollen Index
Vapor Pressure Deficit	Last Year Autumn	−0.463*
	Winter	−0.394
	Last Year Autumn + Winter	−0.469*
	Spring	−0.535**
Sunshine		0.619***
Mean Temperature		0.649***
Maximum Temperature		0.721***
Minimum Temperature		0.497*
Wind Speed		0.532**
Rainfall		−0.494*

Significant levels: $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***).

We also found significant correlations between the spring annual values of *Quercus* total pollen and the mean spring annual values of temperature (mean, maximum and minimum), sunshine, VPD, wind speed and rainfall, being positive for the first four variables and negative for the last one (Table 5). Therefore, the higher or lower *Quercus* pollination intensity during spring months is also related with the climatic conditions of each spring.

On the other hand, there were significant positive correlations between annual values of the spring total pollen (spring pollen index) and the vapor pressure deficit (VPD) during the period prior to flowering (i.e., the previous last year's autumn) (Table 5). This suggests that the more pronounced the dryness, the higher the number of preformed buds (not differentiated into vegetative or floral buds) since bud formation in these woody species occurs in autumn. It is only later (in early spring) that these buds will be transformed into floral meristems as a results of floral induction.

Dry periods were associated with high *Quercus* pollen production periods and vice versa, rainy hydrological years (from October to September) matched low *Quercus* pollen production (1992, 1996, 2000, 2004, 2008, 2011 and 2015). We also observed that an intense peak occurs about every four years, after which the peak levels decrease before rising again approximately four years later (1993, 1997, 2001, 2005, 2009 and 2014) (Table 1). A possible explanation of this observation is that everlasting *Quercus* species alternate between high vegetative activity (growth and production of branches and foils) and high reproductive activity (flowering), as occurs with other everlasting Mediterranean species such as *Olea europaea* L. This phenomenon is known as "production alternance", and usually happens every year or every two years, although, more recently, this has tended to increase to every five years.

As for the pollination temporality, significant and positive correlations were obtained between the accumulated values of sunshine and temperature until the start, peak and end of MPS and their corresponding dates, as well as with their duration. The correlations were higher for the peak date with maximum temperature and sunshine, and for the end of MPS with minimum temperature (Table 4). Furthermore, there was a significant negative correlation between mean temperature in winter and the MPS starting date ($r = -0.524$; $p = 0.009$). This shows that the start of the MPS and the flowering period in *Quercus* species is earlier when the previous winters have been warm. The effect of accumulated temperature on the start of the MPS would be discussed below with the accumulated heat.

The later the MPS peak, the lower its value as can be observed comparing years with early peaks (such as 1997, 1998, 1999, 2001, 2009 or 2011) with those with late peaks (such as 1992, 2002, 2003, 2010, 2012, 2013 or 2014) (Fig. 3, Table 2). The negative correlation detected between the peak value and the accumulated temperature (Table 4) may be due to fact that the later the peak happens (and therefore, lower its value), the more heat will have been accumulated until that date.

Table 6
Comparative and results of the different criteria and methods used in the study of the heat accumulation requirements to trigger the start of the *Quercus* main pollen season. 9 °C = Start of heat accumulation from the date when a mean temperature higher than 9 °C is detected; T.C. = Start of the heat accumulation from the date when the lowest daily mean temperature of winter is detected and in which a trend change in temperature is observed until the start of the MPS; 1 st January = Start of the heat accumulation from 1 st January of this year; $\overline{X}$ = Mean; SD = standard deviation; CV = coefficient of variation.

		9 °C	T.C.	1 st January
Degree-days (°D)	$\bar{x}$ (°C)	315.4	302.7	389
	SD (°C)	114	77.7	56.3
	CV (%)	36.1	25.7	14.5
Heat Requirement (HR)	$\bar{x}$ (°C)	315.4	601.8	796
	SD (°C)	114	148.2	84.1
	CV (%)	36.1	24.6	10.6

Accumulated precipitation does not show any correlation with the variables considered (Table 4). By contrast, in herbaceous species, a positive correlation was obtained between this climatic variable and phenological variables related with flower production and its occurrence (Recio et al., 2010; Aboulaich et al., 2012). However, this would have been due to the herbaceous nature of such species, which means that they are more dependent of the soil water to grow and develop. In the case of *Quercus*, soil water does not influence the development of new inflorescences because they are arboreal in nature and have preformed buds.

3.4. Studies on the accumulated heat required to trigger the beginning of Quercus pollination

Quercus heat accumulation requirements before the start of the main pollen season are shown in Table 6. The different results obtained reflect the different methods and criteria used. The combination of method and criterion which shows the lowest data variability (smallest CV) is the calculation of the heat requirement (HR) from 1st January of each year. The criterion which shows the lowest data variability with all the methods is that which starts accumulating heat from 1st January. The method with the lowest variability with all the criteria is the heat requirement method.

The criterion for selecting the start of the heat accumulating period which shows the biggest data variability is that based on the day when the mean temperature is higher than the 9 °C threshold temperature. The fact that are some days when a sudden changes in temperature to below threshold temperature is detected makes it more difficult to select the day on which the heat accumulation starts and introduces statistical noise in the obtained results.

The criterion involving the date of the lowest mean temperature and change in trend has certain disadvantages even though, it considers the mean temperature when selecting the start of the heat accumulating period. Sometimes the trend change is not very noticeable or does not match the date with the lowest recorded temperature. In this last instance, this criterion leaves it to the researcher to select the date, which introduces a degree of subjectivity into the results.

According to the results obtained and to those of others authors (García-Mozo et al., 2002; Legave et al., 2015), the start of the heat accumulating period is probably close to 1st January. The simplicity involved in applying this criterion reduces the subjectivity which other criteria present.

To test the relationship between the start of the heat accumulating period according to the criteria used and the start of the main pollen season, the number of days from 1st January to the start of the heat accumulating period according to each criterion for each year was correlated with the number of days from 1st January to the start of the

main pollen season using Spearman's correlations. No significant correlations were obtained. This and the large variation coefficients of the other criteria suggest that the 1st January criterion is indeed the most appropriate for calculating heat accumulation.

The obtained results shown in Table 6 suggest that an accumulation of approximately 796 °C beyond the 9 °C threshold temperature after 1st January is necessary to trigger the start of the main pollen season. The threshold temperature was established at 9 °C according to a previous study of *Quercus* pollen in Malaga, in which this temperature showed the best results (García-Mozo et al., 2002). Plant physiological activity begins after this threshold temperature (Jato et al., 2002).

If the accumulated temperature is taken into account without discounting the temperature accumulated below the threshold temperature, the total accumulated temperature increases even if the effective accumulated temperature (which induces the start of the main pollen season) stays constant. That may explain the positive correlation previously detected (Table 4) between the different accumulated temperatures and the start date of the MPS. Every year, an accumulation of approximately 796 °C of effective temperature (beyond the threshold temperature) is necessary to trigger the MPS, but some years the accumulation period is longer because the temperature remains below the threshold temperature. The increase in length of this temperature accumulating period results in a higher accumulated temperature (even if it is only 796 °C of effective temperature and the remaining temperature is "non-effective") and it is expressed as a positive correlation with the start date of the main pollen season.

3.5. Trends in intensity and temporality of Quercus pollination

Trends were calculated by simple linear regressions using the annual intensity data and the *Quercus* anemophilous pollination time data of the last 24 years in Malaga (Fig. 4). The linear models obtained were only significant ($p \leq 0.05$) for the end date of the MPS (whose model suggests that there is a trend for the date to be delayed a day and a half per year) and the length of this period (suggesting that there is a trend for this period to increase by one day per year). Considering all 24 years of the study, no significant trend for the start of the MPS was found. Those results match those of the trends studied by García-Mozo et al. (2010), who observed a general trend for pollination in this taxon to beginning earlier. As regard the pollination intensity, a trend to increase pollen production by 100 grains per year during spring moths was detected, but was significant for a p value slightly higher than 0.05.

However, the trends observed for climatic variables were mostly significant (Fig. 5). The maximum, minimum and mean temperature showed a clear trend to increase their mean annual values (significant at $p \leq 0.001$). That would imply some changes in the flowering time and probably an increase in pollen production in the long term. According to the correlation results shown in Table 5, the trend for the temperature to increase (annually) may be regarded as one of the causes for the increasing trend in anemophilous *Quercus* pollen production in spring. The vapor pressure deficit showed a statistically significant trend to increase. According to the correlation results obtained for annual values of this variable, it may also involve an increase in the pollination intensity. The trend for aridity to increase along with temperature is shown to favor increasing the flowering intensity of the predominant trees in Mediterranean forests. It would be very interesting to study whether there is any trend for vapor pressure deficit to increase in other localities of the south of the Iberian Peninsula and in the western Mediterranean area in general.

It was thought that the temperature increase might change the flowering time in these Mediterranean species, but the start of the flowering period has not changed. We only detected a delay in the end of the flowering period and a slight increase in the flowering period length. It is possible that woody Mediterranean species are adapting to climate change by maintaining their phenological behaviour (which is characterized by a long flowering period). It is possible, too, that the late flowering species are delaying or increasing their flowering period. It would be very interesting to investigate the aerobiology (*ex situ* observations) and flowering phenology (*in situ* observations) of deciduous *Quercus* species which grow in mountainous areas, such as *Quercus faginea* and *Quercus alpestris*.

We conclude that the climatic change effect is mainly recorded in pollination intensity of woody anemophilous species and that these species have adapted their flowering time (phenology) to climate change. It is important to remember that climate change is leading to more arid conditions and that Mediterranean plants are adapted to this macrobioclimate (Mediterranean), which is characterized by a long dry period and high temperatures.

Finally, it should be remembered and emphasized that the study area is a coastal location and that a trend for the percentage of wind coming from the sea to increase was detected (significant at $p = 0.007$). That may occasionally reduce the pollen levels detected. As indicated in

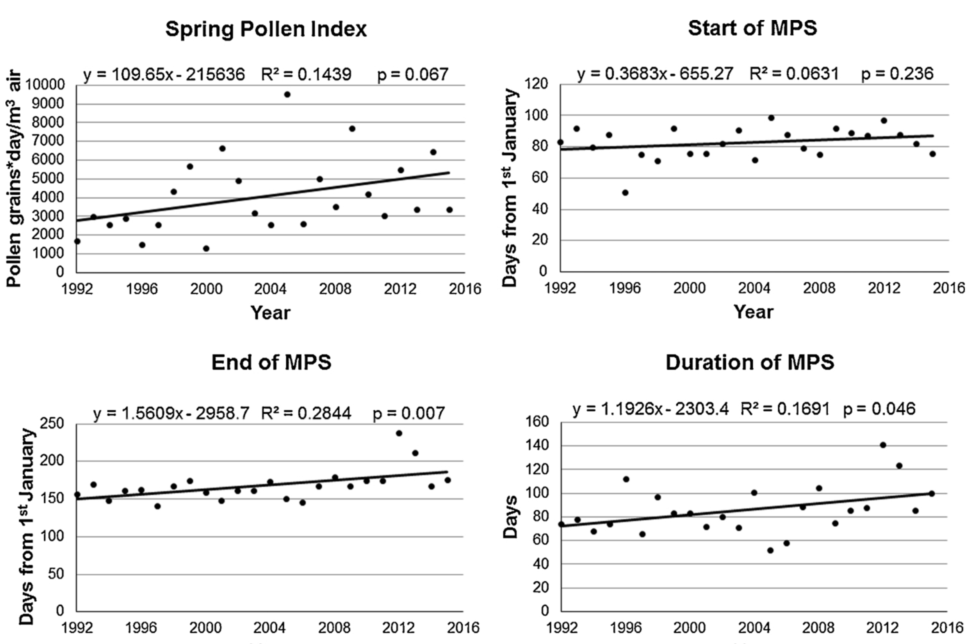

Fig. 4. Linear trends during 1992–2015 for intensity and temporality variables of *Quercus* airborne pollen. Main pollen season (MPS) is 95% of accumulated annual pollen.

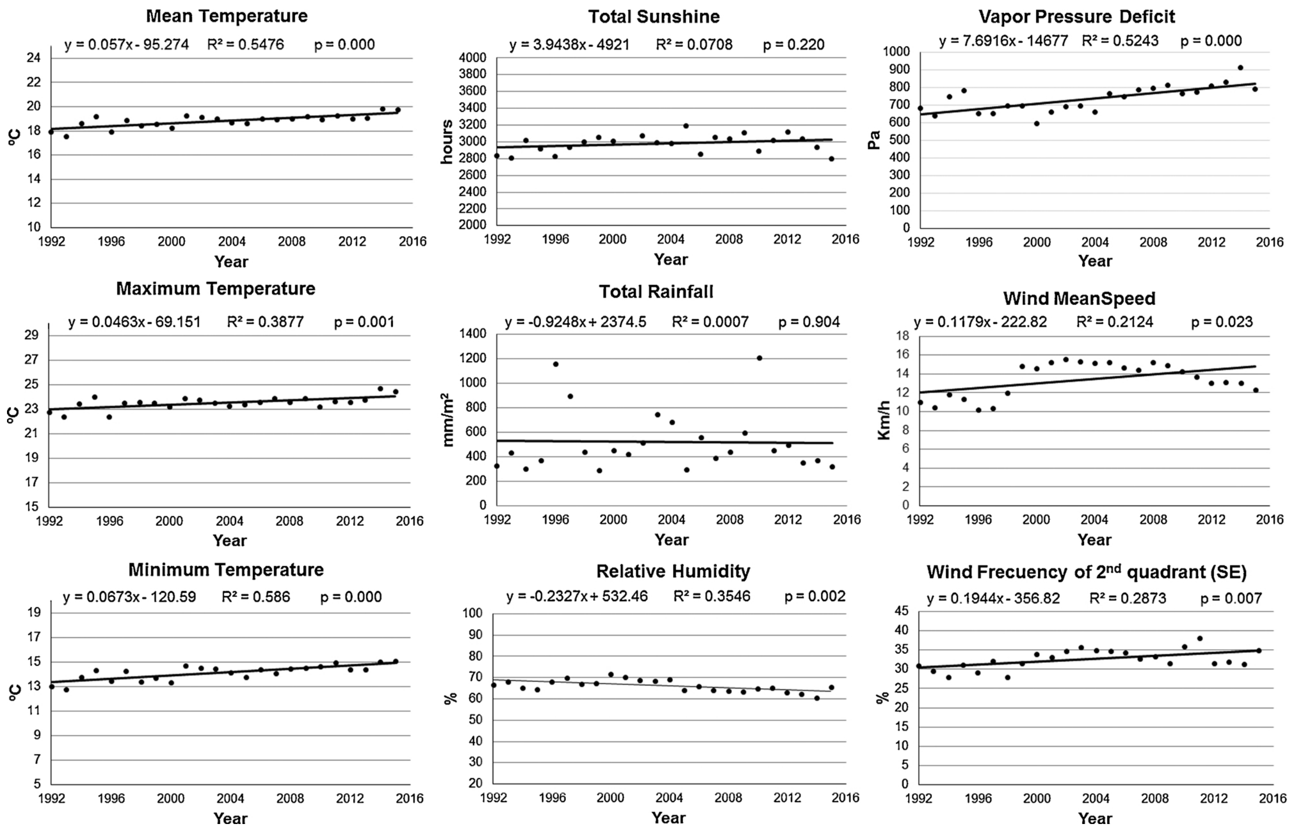

Fig. 5. Linear trends during 1992–2015 for several climatic variables.

a previous study (Recio et al., 2010), it would be very interesting to study whether there is any trend for the mean wind speed to increase in other western Mediterranean locations as in Malaga (significant at $p = 0.02$).

4. Conclusions

1 The annual total register of *Quercus* pollen, which is common in Malaga atmosphere, varied in the period studied (1992–2015). Generally, there has been a high concentration peak every four years. In general, the years of high production in this taxon matched dry periods and *vice versa* (the years with low pollen production match the rainy hydrological years).

2 Most (98%) of the *Quercus* pollen in the Malaga atmosphere is recorded in March, April, May and June (from the end of winter until the end of spring) and the weekly data may show one, two or three (rarely more) peaks. In the studied period (1992–2015) the earliest annual maximum peaks were produced at the end of March, and the latest at the end of May. Pollination period length also changes each year, varying from little more than a month to three months.

3 Fluctuations in atmospheric concentrations of *Quercus* pollen in Malaga (daily and weekly values) are significantly and positively associated with temperature and insolation changes, but negatively associated with precipitation and relative humidity. They are also significantly and positively associated with wind speed and northwestern wind frequency.

4 The annual intensity of anemophilous pollination of *Quercus* is significantly associated with the climatic conditions of each spring, with the same parameters involved and in the same way as in the daily and weekly scale. Moreover, it is positively associated with the vapor pressure deficit of the previous autumn, which probably influences the production of preformed and undifferentiated buds. These undifferentiated buds will later turn (in spring) into reproductive buds, which would produce flowers. The tendency for the temperature and atmosphere aridity to increase is probably the cause of the trend for the spring *Quercus* pollen production to increase in the western Mediterranean.

5 The temporality of *Quercus* anemophilous pollination (start date, peak date, end date and duration) is positively associated with accumulated temperature and sun hours from 1st January until to the dates in question. An accumulation of approximately 796 °C above the 9 °C threshold temperature since 1st January is necessary to trigger the start of the flowering period. Hot winters are associated with an earlier start of the flowering period.

6 There was no any significant trend for the start of *Quercus* pollination between 1992 and 2015, and only slight trends for the end to be delayed and the pollination period to increase were detected. It is possible that woody perennial *Quercus* species, which are widely distributed in Mediterranean macrobioclimate areas (characterized by long dry periods and hot temperatures), are adapting their phenological behaviour (longer duration of flowering) and possibly their geographical distribution (increasing in altitude) as a consequence of climate change.

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